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cont. SPI-GHRE. Subsequently the extracts were analysed on polyacrylamide gels. The results showed that nuclear proteins, dependent on GH, bound to this DNA sequence. By competition with shorter oligonucleotides derived from SPI-GHRE a core GH sequence was identified. Based on certain sequence homologies to interferon response-elements we called this sequence SPI-GAS and also demonstrated that SPI-GAS functions as a GH regulated DNA element when put into a reporter vector. The core SPI-GAS has the following sequence; TTCTGAGAA.

Example 2. Prolactin and growth hormone both activate SPI-TK reporter gene.

An expression plasmid containing a recombinant hormone responsive reporter consisting of six repeats of a 52 bp growth hormone responsive element (GH-RE) from the serine protease inhibitor (SPI) 2.1 promoter fused to the thymidine kinase (TK) promoter was constructed. Corresponding constructs were made using the SPI-GAS element. Variants expressing either the bacterial protein chloramphenicol acetyl transferase (CAT) or firefly luciferase (SPI-CAT or SPI-Luc respectively) cDNAs were then constructed. Techniques to make these vectors are well known to experts in the field. The plasmid DNA constructions were transfected, together with plasmid expression vectors encoding either rat growth hormone receptors or mouse prolactin receptors, into Chinese hamster ovary (CHO), COS, and Buffalo rat liver (BRL) cells, using DOTAP liposomes and according to the manufacturer instructions. Cells were incubated overnight with DNA and DOTAP in serum free media, left and then exposed to growth hormone or prolactin for 12 hours. Cell lysates were then prepared and CAT or luciferase enzyme activity measured. Both growth hormone and prolactin treatment lead to an approximately 5-fold stimulation reporter enzyme expression relative to transfected but non-hormone treated cells. These results show that both growth hormone and prolactin can regulate the reporter construct and that a requisite for this is the presence of SPI elements. The core element in the SPI-TK-reporter gene that confers GH